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MINIREVIEW

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The path from retrovirus to cellular oncogene is a common one. All retroviral oncogenes are derived from the genome of the host cell. They are mutated and assimilated into the viral genome. Their replication, expression, and movement into new cells are all under viral control (for reviews see Bishop, 1983; Varmus, 1984). As genetically altered captives, they reveal latent properties that are not evident in their cellular progenitors. The *qin* gene is a recent example, discovered as a cell-derived oncogenic determinant in avian sarcoma virus 31 (ASV 31) (Li and Vogt, 1993). The genome of ASV 31 is similar to that of other defective transforming retroviruses (Fig. 1). Part of the viral *gag* and all of the *pol* region are missing and replaced by the *qin* insert that is expressed as a Gag–Qin fusion protein. The *qin* or *gag–qin* sequences excised from the ASV 31 genome and inserted into the retroviral expression vector RCAS show the same oncogenic potential as the native ASV 31. Therefore, *qin* functions as the oncogenic determinant of ASV 31; it is its oncogene (Chang *et al.,* 1996; Li and Vogt, 1993).

Qin codes for a protein that belongs to the winged helix family of transcription factors. The hallmark of this family is a conserved DNA binding domain, about 100 amino acids in length (Lai *et al.,* 1993; Kaufmann and Knöchel, 1996). One of the three α helixes in this domain makes contacts with the major groove of double-stranded DNA. These contacts are stabilized by adjacent loop structures that also interact with the DNA, hence the term "winged helix" (WH) (Brennan, 1993; Clark *et al.,* 1993). Outside the DNA binding domain, WH proteins show considerable diversity of sequence and structure. They function prominently as determinants of organ development and tissue differentiation (Costa, 1994). The prototype gene coding for a WH protein was discovered during developmental studies in *Drosophila;* its name, forkhead (fkh), refers to the phenotype of mutants that show homeotic transformations in the anterior and posterior gut with head-like structures at both ends of the embryo (Weigel *et al.,* 1989). The developmental regulator hepatocyte nuclear factor 3β (HNF-3β) was one of the first WH proteins identified in mammals (Lai *et al.,* 1991). It is highly expressed in the node, notochord, floor plate, and endoderm of the mouse embryo. HNF-3β null mutations cause embryonic lethality with defects in node and notochord formation (Ang and Rossant, 1994; Weinstein *et al.,* 1994). More than half a dozen WH genes have now been analyzed by germ line knockouts, and all of these show informative phenotypes (Ang and Rossant, 1994; Dou *et al.,* 1997; Hatini *et al.,* 1996; Labosky *et al.,* 1997; Weinstein *et al.,* 1994; Winnier *et al.,* 1997; Xuan *et al.,* 1995). A very familiar example of a developmentally important WH gene is the nude mouse, whose immunological deficiencies and lack of hair result from a mutation in the *whn* gene that codes for a WH transcription factor (Nehls *et al.,* 1994).

Qin is the homolog of the mammalian brain factor 1 (BF-1). The expression of BF-1 is restricted to the telencephalon, the nasal half of the retina, and the optic stalk (Tao and Lai, 1992). In the developing chicken embryo expression of Qin is also confined to the telencephalon (Chang *et al.,* 1995). Mice with null mutations of BF-1 die at birth with severe defects in the development of the cerebral hemispheres (Xuan *et al.,* 1995). BF-1 is thought to control the replication of cortical progenitor cells, but the mechanism of this regulation is not understood (McConnell, 1995). Overexpression of Qin from a retroviral vector in the developing avian retina distorts the visual projection map on the optic tectum, presumably by interfering with normal mechanisms of axon guidance (Yuasa *et al.,* 1996). Recently described continuous cell lines of murine neocortical neuroblasts show high expression of BF-1 (Chun and Jaenisch, 1996). Although these observations and data on the role of BF-1/Qin are incomplete and fragmentary, they are clear indicators that we are dealing with an important control element in brain devel-

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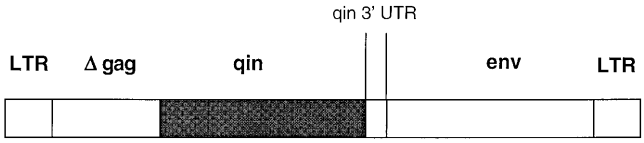

FIG. 1. Map of the ASV 31 genome. Δgag and env represent viral coding sequences of the genes for internal structural proteins and envelope proteins, respectively. Qin is a cell-derived insert that replaces the 3′ portion of the *gag* and all of the *pol* gene. LTR, long terminal repeat; UTR, untranslated region.

opment. Further experiments on BF-1/Qin in embryogenesis will be both interesting and challenging and will help us understand the oncogenicity of the gene.

The viral Qin protein (v-Qin) differs from its cellular counterpart both structurally and functionally (Chang *et al.,* 1995; Li *et al.,* 1997) (Fig. 2). Its amino terminus is fused to Gag sequences with an eight-amino-acid linker, probably of cellular origin. v-Qin is also truncated at the carboxyl terminus by a premature stop codon, and the last eight amino acids of v-Qin are not homologous to cellular Qin (c-Qin). In addition, the coding sequence of v-Qin shows two nonconservative amino acid substitutions: a G to D substitution in the DNA binding domain and an S to N substitution near the amino terminus in a presumed transcription regulatory region.

Both c-Qin and v-Qin proteins, expressed from the retroviral vector RCAS, stimulate the growth of chicken embryo fibroblasts (CEF), inducing the formation of transformed cell foci and of agar colonies in cell culture (Li *et al.,* 1997). v-Qin is also highly tumorigenic in the animal in contrast to c-Qin, which causes tumors in only a small fraction of the injected animals and only after a prolonged latent period (Li *et al.,* 1997). It is possible that these rare c-Qin-induced tumors arise as a result of mutations in the oncogene that is being expressed from an actively replicating and mutation-prone retroviral vector. Qin proteins are localized in the nucleus (Li and Vogt, 1993). They bind to the consensus sequence TGTAAACAAA. c-Qin binds to this target sequence with far greater affinity than v-Qin (Li *et al.,* 1995). The difference has been mapped to the G to D substitution in the DNA binding domain. This mutation seems to weaken v-Qin DNA interaction in general, because it has not been possible to identify a variant high-affinity target sequence of v-Qin by PCR selection. A D to G substitution introduced onto a v-Qin background makes the protein less oncogenic in the animal. The fact that tumor production by this point mutation is reduced but not abolished suggests that other mutations present in v-Qin contributes to oncogenicity (Li, Chang, Thurm, and Vogt, 1996, unpublished observation).

In transient transfection tests with a luciferase reporter carrying six Qin binding sites, both Qin proteins function as repressors (Li *et al.,* 1995). Repression is also observed if Qin is linked to the GAL4 DNA binding domain and reporter plasmids carry GAL4 binding sites. Deletion of the GAL4 target sequences on the reporter then abolishes Qin-induced transcriptional repression. Binding to DNA is therefore required for this repression effect, which may be caused by competition with an activator for the same DNA binding site, by a protein–protein interaction of Qin with a transcriptional activator (quenching), or by an interaction of Qin with a component of the basic transcriptional machinery (direct repression). The latter two mechanisms require a specific repressor domain separate from the DNA binding domain. Such a domain has been identified in Qin; like the repressor domains of some known direct repressor proteins, it is rich in alanines. v-Qin is a stronger repressor than c-Qin, which appears paradoxical considering its reduced affinity for DNA (Li *et al.,* 1997). However, an inverse relationship between DNA binding and transcriptional repression is not unique for Qin. It has been observed with other repressors, for instance *Drosophila* Even-skipped (Han and Manley, 1993). One suggested explanation for this fact is that the repressor binds its DNA target and then contacts a component of the transcription initiation complex, thus interfering with transcription. The interaction between repressor and transcriptional machinery is facilitated by a dissociation of repressor and DNA (Han and Manley, 1993).

Transcriptional repression and oncogenic transformation have been mapped by deletion analysis to overlapping domains in Qin: both activities depend on (in addition to the intact WH region) sequences in the carboxyl-terminal third of the molecule (Li *et al.,* 1995). This colocalization of repression and transformation domains suggests a possible role of transcriptional repression in oncogenicity. The suggestion is strengthened by the properties of chimeras in which the carboxyl-terminal repression domain of Qin has been replaced either with the transactivation domain of the herpes simplex virus protein VP16 or the repression domain of the *Drosophila* protein Engrailed (Poole *et al.,* 1985; Triezenberg *et al.,* 1988). Qin–Engrailed chimeras function as repressors; they transform CEF in cell culture and induce tumors in the animal. Qin–VP16 chimeras activate transcription from Qin binding sites; they are not oncogenic *in vitro* or *in vivo.* The cells expressing Qin–VP16 are flat and highly

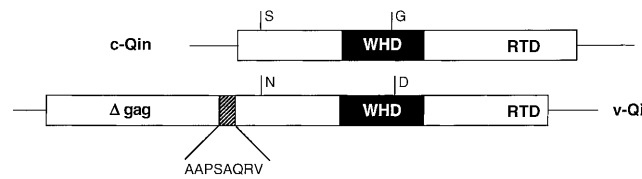

FIG. 2. c-Qin and v-Qin proteins compared. v-Qin is fused to Gag via a cell-derived seven-amino-acid linker. It has two amino acid substitutions: a S → N in the amino-terminal half and a G → D in the DNA-binding region. WHD, winged helix domain; RTD, repression and transformation domain.

adherent, and they show a 10- to 50-fold resistance to transformation with v-Qin.

The correlation between transcriptional repression and oncogenicity and the colocalization of transforming and repression domains on the genetic map of Qin suggest that Qin induces oncogenic transformation by downregulating the expression of specific genes. Determining the identity and function of these genes remains a major and urgent task for the future. In general terms, direct Qin targets that control the oncogenic phenotype of the cell would have to be negative growth regulators and thus tumor suppressor genes. Two of the possible mechanisms of Qin-dependent transcriptional repression, quenching and direct repression, require protein–protein interactions between Qin and either an activator protein or a component of the transcription initiation complex. Defining and characterizing these interactions is a second important challenge for the future.

Numerous WH proteins have been described over the past 6 years (Kaufmann and Knöchel, 1996), and it is reasonable to assume that Qin is not the only one that has latent oncogenic properties. Since transcription factor oncogenes (with the only exception of *fos*) are much more effective in transforming avian than mammalian cells, a search for oncogenic WH proteins commenced with the chicken genome. The cDNA clones "chicken winged helix (CWH) 1, 2, and 3" were isolated from a chicken embryonic cDNA library by low-stringency hybridization using the sequence that codes for the c-Qin DNA binding domain as a probe (Freyaldenhoven *et al.,* 1997a). Comparison with the genetic sequence database showed CWH-1 to be most closely related to mouse brain factor 2 (BF-2) and to its rat homolog HFH-B2 (Clevidence *et al.,* 1993; Hatini *et al.,* 1994). It is also closely related to the human gene termed forkhead-related activator 4 (FREAC-4) (Pierrou *et al.,* 1994). CWH-2 is most likely the chicken homolog of the mouse mesenchyne forkhead protein MFH-1 (Miura *et al.,* 1993). CWH-3 shares significant homology with the rat HNF-3/fkh homolog HFH-2 (Clevidence *et al.,* 1993) and with a recently identified mouse winged helix factor Genesis (Sutton *et al.,* 1996). All three CWH proteins show tissue-specific expression by Northern blot analysis. CWH-1 mRNA is found in embryonic brain, with weaker signals in intestine and kidney. CWH-2 mRNA is detectable in embryonic and adult brain and kidney. CWH-3 mRNA is restricted to embryonic lung and intestine and is not detectable in adult tissues. In CEF, CWH-1 and CWH-2 but not CWH-3 mRNAs are observed.

In electrophoretic mobility shift assays, the CWH proteins bind with different affinities to two conserved DNA binding sites for WH proteins, the B2 site in the HNF-1 promoter (Kuo *et al.,* 1992) and the Qin binding site (Freyaldenhoven *et al.,* 1997b; Li *et al.,* 1997). CWH-1 and CWH-2 also bind to the TTR site in the transthyretin promoter (Costa *et al.,* 1989). Overexpression of wildtype CWH-1, CWH-2, and CWH-3 proteins from the replication-competent retroviral vector RCAS induces changes in morphology and growth pattern of CEF (Freyaldenhoven *et al.,* 1997a). CWH-1 and CWH-3 stimulate anchorage-independent growth and cause increased saturation density of CEF. Wildtype CWH-2 is less growth stimulatory, but can mutate to induce foci of transformed cells (Vogt, 1997, unpublished observation). These results suggest that the potential to stimulate abnormal cell proliferation is common among WH proteins.

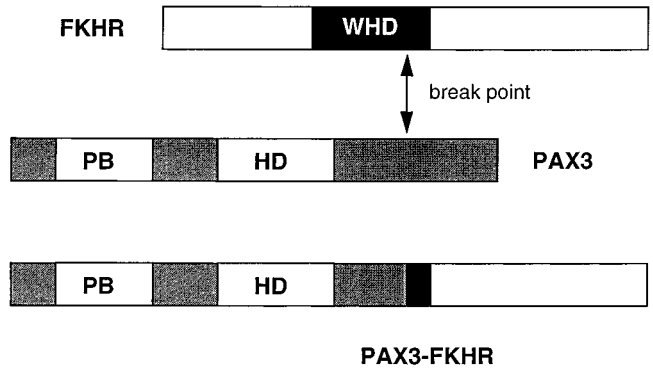

FIG. 3. Fusion of PAX3 and FKHR resulting from the recurrent t(2;13) translocation in alveolar rhabdomyosarcoma. WHD, winged helix domain; PB, paired box; HD, homeodomain. The fusion protein retains the DNA binding domain of PAX3 but not that of FKHR. The carboxy-terminal portion of FKHR may contain a strong transactivation domain.

Similar to Qin, CWH-1, CWH-2, and CWH-3 function as transcriptional repressors in CEF, either as wildtype proteins acting on a reporter with a Qin binding site or as GAL4 fusion products acting on a reporter containing GAL4 binding sites (Freyaldenhoven *et al.,* 1997b). The strongest repressor in these tests is CWH-3. Its repression domain was mapped to a 96-amino-acid sequence in the carboxyl-terminal third of the protein. This region is homologous to the repression domain of Qin in position and amino acid composition. Both repression domains share a high content of proline or alanine residues, which are characteristic features of repression domains found in prototypical direct transcription repressors (Cowell, 1994; Hanna-Rose and Hansen, 1996). The role of transcriptional repression in the growth regulatory activities of CWH-1, CWH-2, and CWH-3 remains to be determined.

Oncogenic WH proteins have been uncovered by yet another line of investigation: the study of chromosomal rearrangements that occur with high regularity and frequency in certain human cancers, notably leukemias, lymphomas and some solid tumors. In pediatric alveolar rhabdomyosarcoma, a recurrent t(2;13)(q35q14) translocation generates a chimeric product that contains the amino-terminal portion of the PAX3 protein fused to the carboxyl-terminal region of the winged helix protein FKHR (Barr *et al.,* 1993; Galili *et al.,* 1993; Shapiro *et al.,* 1993) (Fig. 3). In this fusion product, FKHR is truncated

within the winged helix domain, but still contains a putative transactivation domain at its carboxyl terminus. The PAX3-derived portion encompasses the intact paired box and homeodomains and provides the sequence-specific DNA binding function of the fusion protein. A variant t(1;13)(p36q14) chromosomal translocation found in a subset of alveolar rhabdomyosarcomas fuses the amino-terminal portion of the PAX7 protein to the same carboxyl-terminal FKHR fragment as in the prevalent t(2;13) translocation (Biegel *et al.,* 1991; Douglass *et al.,* 1991; Whang-Peng *et al.,* 1992). Again, DNA binding is determined by the PAX component (Davis *et al.,* 1994). The *FKHR* gene contains three exons (Davis *et al.,* 1995). Exon 1 extends to and includes the amino-terminal region of the winged helix domain. The carboxyl-terminal region of the winged helix domain and a transcriptional activation domain are encoded by exon 2, while exon 3 consists of the 3′ untranslated region. The boundary between intron 1 and exon 2 coincides with the *FKHR* fusion point in the chimeric transcripts found in alveolar rhabdomyosarcomas (Davis *et al.,* 1995). PAX3 and PAX7 proteins code for transcriptional regulators that control developmental programs. They share similar expression patterns in the developing nervous system as well as in the developing somites of the mammalian embryo around the time of dermomyotome formation (Bober *et al.,* 1994; Chalepakis *et al.,* 1993; Goulding *et al.,* 1991, 1994; Jostes *et al.,* 1991; Williams and Ordahl, 1994). PAX3 expression occurs prior to myoblast migration and formation of the musculature; PAX7 expression begins a few days later and is maintained during the differentiation of the trunk and limb muscles. Both PAX3 and the PAX3–FKHR fusion protein can prevent myogenic differentiation of cultured myoblast cells (Epstein *et al.,* 1995). Compared to PAX3, the fusion product PAX3–FKHR is a stronger activator of transcription and inhibitor of myogenic differentiation (Fredericks *et al.,* 1995). Overexpression of the PAX3–FKHR fusion protein from the retroviral RCAS vector induces morphological transformation and anchorage-independent growth of CEF in culture. Wildtype PAX3 expressed from the same vector does not transform CEF (Scheidler *et al.,* 1996). The fact that the PAX3–FKHR protein is a more potent transcriptional activator than wildtype PAX3 suggests that the transforming activity of the fusion protein may reflect a gain of activating function on PAX3 targets. Downregulation of the PAX3–FKHR fusion gene product in alveolar rhabdomyosarcoma cells by antisense oligonucleotides induces cellular apoptosis (Bernasconi *et al.,* 1996).

These data suggest that the *PAX–FKHR* fusions may act as dominant oncogenes responsible for the initiation and maintenance of the neoplastic phenotype in alveolar rhabdomyosarcoma cells. They may aberrantly regulate the transcription of *PAX* target genes, and this differential regulation of gene expression may result in tumor formation, perhaps by suppressing an apoptotic program that would normally eliminate some of these embryonic cell lineages.

The second winged helix transcription factor shown to be rearranged in human cancer is *AFX.* It was found as a fusion partner of the *MLL* gene (also referred to as *ALL* or *HRX*). The fusion results from t(X;11)(q13q23) translocations in acute lymphocytic leukemia and in nonlymphoblastic leukemia (Borkhardt *et al.,* 1997; Corral *et al.,* 1993; McCabe *et al.,* 1994; Parry *et al.,* 1994). The *MLL* gene maps to q23 on chromosome 11. It participates in numerous diverse translocations in acute leukemias (Rabbitts, 1994; Rowley, 1993). In these rearrangements, the 5′ region of *MLL* is fused with different partner genes, some of which have been identified and genetically characterized (McCabe *et al.,* 1992; Parry *et al.,* 1993; Rowley, 1993). *MLL* fusion partners commonly contain structural motifs that are also seen in transcriptional regulators such as zinc-fingers and leucine-zippers in the *AF10* and *AF17* genes or in nuclear targeting sequences in *AF4* (Chaplin *et al.,* 1995; Nakamura *et al.,* 1993; Prasad *et al.,* 1994). The *MLL* protein is related to the trithorax protein of *Drosophila.* The two show high homology in their carboxyl-terminal portion and share similar zinc-finger motifs (Djabali *et al.,* 1992; Gu *et al.,* 1992; Tkachuk *et al.,* 1992). The *AFX* gene is homologous to the *FKHR* winged helix transcription factor of human alveolar rhabdomyosarcoma (Borkhardt *et al.,* 1997). The fusion point in the MLL–AFX1 protein is at the same amino acid position within the winged helix domain as in the PAX3–FKHR fusion, rendering the DNA binding domain of AFX1 nonfunctional (Parry *et al.,* 1994). The AFX protein contains some potentially interesting motifs, including a hexapeptide that is highly homologous to one seen in Hox proteins, where it mediates cooperative DNA binding of the Hox–Pbx complex. This hexapeptide is missing in the MLL–AFX1 fusion product. The biochemical functions of the MLL–AFX protein remain to be determined.

v-*qin* functions as a dominantly acting oncogene, and the fusion genes *PAX3–FKHR* and *MLL–AFX* may play a similar role in human cancer. v-*qin* and *PAX3–FKHR* have properties of transcriptional regulators. They bind sequence specifically to DNA and stimulate or repress the transcription of reporter genes. They may, therefore, induce oncogenic transformation by aberrant positive or negative regulation of specific target genes. Although the precise mechanism of action for *MLL–AFX* remains to be worked out, this fusion product also contains elements of a transcriptional regulator. Transcriptional regulators are themselves controlled at transcriptional and posttranscriptional levels. All WH proteins studied so far show characteristic developmental stage- and tissue-specific expression. Little is known about the upstream signals that are in charge of these expression patterns.

The oncogenicity of WH proteins is part of a broader problem, the oncogenicity of transcriptional regulators in general. The important questions are the same through-

out this field, i.e., identification of downstream target genes and their function in inducing the oncogenic cellular phenotype, characterization of protein–protein interactions that stimulate or inhibit the transcriptional machinery, and clarification of upstream signals that control the transcriptional regulator. The technical advances in the isolation of differentially expressed genes and in the detection of protein–protein interactions continue to be rapid and promise to yield insights into both viral and nonviral oncogenesis mediated by aberrant transcriptional control (Aronheim *et al.,* 1997; Lavery *et al.,* 1997; Shalon *et al.,* 1996).

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